

AMENDMENTS

Amendments to the Claims

Please amend the claims according to the following listing of the claims.

Listing of the claims

1. (withdrawn) A cytochrome P450 monooxygenase which is capable of at least one of the following reactions:
 - a) oxidation of optionally substituted N-, O- or S-heterocyclic mono- or polynuclear aromatic compounds;
 - b) oxidation of optionally substituted mono- or polynuclear aromatics;
 - c) oxidation of straight-chain or branched alkanes and alkenes;
 - d) oxidation of optionally substituted cycloalkanes and cycloalkenes;

where the monooxygenase is derived from cytochrome P450 monooxygenase BM-3 from *Bacillus megaterium* having an amino acid sequence according to SEQ ID NO:2, which has at least one functional mutation in at least one of the amino acid sequence regions 172-224, 39-43, 48-52, 67-70, 330-335, 352-356, 73-82 and 86-88; except the single mutant Phe87Val.

2. (withdrawn) A monooxygenase as claimed in claim 1, which has at least one functional mutation in at least one of the sequence regions 73-82, 86-88 and 172-224.
3. (withdrawn) A monooxygenase as claimed in claim 1,

which has at least one of the following mono- or polyamino acid substitutions:

- a) Phe87Val, Leu188Gln; or
- b) Phe87Val, Leu188Gln, Ala74Gly;

and functional equivalents thereof which are capable of at least one of the above oxidation reactions.

- 4. (withdrawn) A nucleic acid sequence coding for a monooxygenase according to claim 1.
- 5. (withdrawn) An expression construct comprising, under the genetic control of regulatory nucleic acid sequences, a coding sequence which comprises a nucleic acid sequence according to claim 4.
- 6. (withdrawn) A vector comprising at least one expression construct according to claim 5.
- 7. (withdrawn) A recombinant microorganism transformed by at least one vector as claimed in claim 6.
- 8. (withdrawn) A microorganism as claimed in claim 7, selected from bacteria of the genus *Escherichia*.
- 9. (currently amended) A process for the microbiological oxidation of an N- or S-heterocyclic mono- or polynuclear aromatic compound which comprises
 - a1) culturing a recombinant microorganism which expresses a cytochrome P450 monooxygenase of

- bacterial origin in a culture medium, in the presence of an exogenous or intermediately formed substrate; or
- a2) incubating a substrate-containing reaction medium with a cytochrome P450 monooxygenase of bacterial origin; and
 - b) isolating the oxidation product formed or a secondary product thereof from the medium, and

wherein the monooxygenase is derived from cytochrome P450 monooxygenase BM-3 from *Bacillus megaterium* having ~~an~~ the amino acid sequence according to SEQ ID NO: 2, which has at least one functional mutation in at least one of the amino acid sequence regions 172-224, 39-43, 48 - 52, 67-70, 330-335, 352-356, 73-82 and 86-88.

- 10. (currently amended) A process as claimed in claim 9, wherein the exogenous or intermediately formed substrate of claim 9, alternative a1), or the substrate contained in the reaction medium of claim 9, alternative a2) is selected from optionally substituted N- or S-heterocyclic mono- or polynuclear aromatic compounds.
- 11. (canceled)
- 12. (previously presented) A process as claimed in claim 9, where the mutant has one of the following mono- or polyamino acid substitutions:
 - a) Phe87Val;

- b) Phe87Val, and Leu188Gln;
 - c) Phe87Val, and Leu188Gln, and Ala74Gly.
13. (withdrawn) A process for microbiological oxidation of optionally substituted mono- or polynuclear aromatics, straight-chain or branched alkanes or alkenes, or optionally substituted cycloalkanes or cycloalkenes, which comprises
- a1) culturing a recombinant cytochrome P450-producing microorganism as claimed in claim 7 in a culture medium, in the presence of an exogenous or intermediately formed substrate; or
 - a2) incubating a substrate-containing reaction medium with a cytochrome P450 monooxygenase derived from cytochrome P450 monooxygenase BM-3 from *Bacillus megaterium* having an amino acid sequence according to SEQ ID NO:2, which has at least one functional mutation in at least one of the amino acid sequence regions 172-224, 39-43, 48-52, 67-70, 330-335, 352-356, 73-82 and 86-88; and
 - b) isolating the oxidation product formed or a secondary product thereof from the medium;

where the monooxygenase mutant Phe87Val is not excluded.

14. (withdrawn) A process as claimed in claim 13, wherein the exogenous or intermediately formed substrate is selected from:
- a) optionally substituted mono- or polynuclear

- aromatics;
 - b) straight-chain or branched alkanes and alkenes;
 - c) optionally substituted cycloalkanes and cycloalkenes.
15. (canceled)
16. (withdrawn) A process as claimed in claim 13, where the cytochromeP450 monooxygenase has at least one of the following mono- or polyamino acid substitutions:
- a) Phe87Val;
 - b) Phe87Val, Leu188Gln; or
 - c) Phe87Val, Leu188Gln, Ala74Gly.
17. (previously presented) A process as claimed in claim 9, wherein, as exogenous substrate, at least one compound selected from unsubstituted or substituted N-, O- or S-heterocyclic mono- or polynuclear aromatic compounds is added to a medium and the oxidation is carried out by enzymatic reaction of the substrate-containing medium in the presence of oxygen at a temperature of approximately 20 to 40°C and a pH of approximately 6 to 9, where the substrate-containing medium additionally contains an approximately 10- to 100-fold molar excess of reduction equivalents based on the substrate.
18. (previously presented) A process as claimed in claim 17, wherein, as exogenous substrate, a compound selected from indole, 1-methylindole, acridine, 6-methyl- or 8-methylquinoline, quinoline and quinaldine

is employed.

19. (withdrawn) A process for the microbiological production of indigo and/or indirubin, which comprises
 - a1) culturing a recombinant microorganism which produces an indole-oxidizing cytochrome P450 in a culture medium, in the presence of exogenous or intermediately formed indole; or
 - a2) incubating an indole-containing reaction medium with an indole-oxidizing cytochrome P450 monooxygenase; and
 - b) isolating the oxidation product formed or a secondary product thereof from the medium.
20. (withdrawn) A process as claimed in claim 19, wherein the indigo and/or indirubin obtained, which was produced by oxidation of intermediately formed indole, is isolated from the medium.
21. (withdrawn) A process as claimed in claim 20, wherein the indole oxidation is carried out by culturing the microorganisms in the presence of oxygen at a culturing temperature of approximately 20 to 40°C and a pH of approximately 6 to 9.
22. (withdrawn) A process as claimed in claim 20, where the monooxygenase is derived from cytochrome P450 monooxygenase BM-3 from *Bacillus megaterium* having an amino acid sequence according to SEQ ID NO:2, which has at least one functional mutation in at least one of the amino acid sequence regions 172-224, 39-43, 48-

52, 67-70, 330-335- 352-356, 73-82 and 86-88,
including the substitution Phe87Val.

23. (withdrawn) A process as claimed in claim 22, where the monooxygenase has at least one of the following mono- or polyamino acid substitutions:
- a) Phe87Val;
 - b) Phe87Val, Leu188Gln; or
 - c) Phe87Val, Leu188Gln, Ala74Gly.
24. (withdrawn) A bioreactor comprising the cytochrome P450 monooxygenase as claimed in claim 1 or a recombinant microorganism transformed by a vector comprising an expression construct comprising a nucleic acid sequence coding for the cytochrome P450 monooxygenase of claim 1 in immobilized form.
25. (canceled)
26. (canceled)